Use of *in vivo*-induced antigen technology to identify bacterial genes expressed during Sol*ea senegalensis* infection with *Photobacterium damselae* subsp. *piscicida*

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Abstract

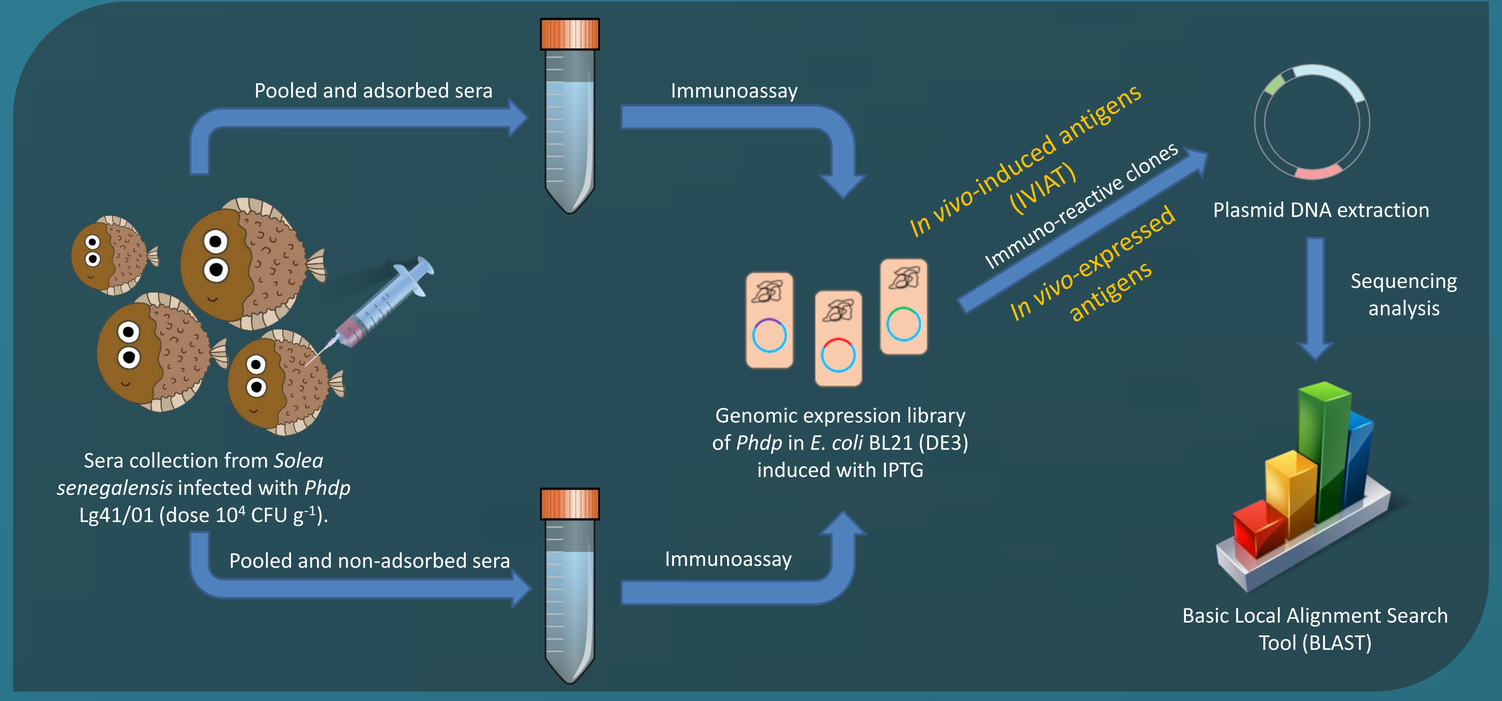
The marine fish pathogen *Photobacterium damselae* subsp. *piscicida* (*Phdp*) is responsible for important outbreaks affecting several fish species including flatfish *Solea senegalensis*. The aim of this work was to identify *in vivo*-induced expressed immunogenic proteins using pooled sera from fish that have experienced photobacteriosis. *In vivo* induced immunogenic proteins included inosine-5'-monophosphate dehydrogenase (Impdh) and alkyl hydroperoxide reductase (AhpC), two proteins involved in peptide synthesis: serine hydroxymethyl transferase (Shmt) and alanyl-tRNA synthetase (AlaRS) and the non-ribosomal peptide synthetase involved in the synthesis of the siderophore piscibactin (Irp2).

Introduction

Photobacterium damselae subsp. *pisicicida* (*Phdp*) is an opportunist pathogen in marine fish responsible for important economic losses. Several virulence factors have been identified in Phd; however, most studies have been carried out *in vitro* and bacterial activities are modulated by their environment. Genes expressed during pathogen infection are important for pathogenicity. In vivo-induced antigen technology (IVIAT) has been used to identify in vivo-induced genes using pooled sera from fish that have experienced photobacteriosis.

Materials and Methods

Sera were obtained from *S. senegalensis* specimens after sublethal infection with *Phdp* and subsequently pooled and adsorbed against *in vitro* grown *Phdp* Lg41/01 and *Escherichia coli* BL21 (DE3) cells and lysates to remove antibodies against *in vitro* expressed antigens according to Handfield et al. [1]. The efficiency of sera adsorption was evaluated by ELISA, based on the immuno-reactivity with whole and lysed *Phdp* cells, grown *in vitro,* as immobilized antigens. A genomic expression library of *Phdp* Lg41/01 was generated in *E. coli* BL21 (DE3) using pET-30 expression system (Novagen, San Diego, CA, USA). The expression library was probed with adsorbed (for *in vivo*-induced antigens, IVIAT) and non-absorbed sera (for *in vivo*–expressed antigens) using immunoblot technique. Inserted DNA from reactive clones was sequenced (Macrogen Europe, Amsterdam, The Netherlands). Nucleotide sequences were compared against the NCBI protein database using BLASTx.



Results

Specific antibody titers against *Phdp* were determined in the sera from each surviving *S. senegalensis* specimen. All fish showed significantly higher antibody titers compared to control fish (non-infected fish). After sera adsorption rounds, a progressive reduction in sera immuno-reactivity against *in vitro* grown *Phdp* cells was detected, especially after the first adsorption step. Thus, following adsorption steps substantially resulted in relative enrichment in antibodies recognizing *in vivo* expressed antigens. The library from *Phdp* Lg14/01 constructed in *E. coli* BL21 (DE3) consisted of approximately 6500 recombinants.

Functional category	Identification	Accession	Predicted cellular location
Replication, recombination and repair	DNA gyrase subunit B	WP_044180341.1	Cytoplasm
	Recombination associated protein RdgC	WP_044176475.1	Cytoplasm
Iron acquisition	Non-ribosomal peptide synthetase	AKQ52531	Membrane
Transport and metabolism	Inosine-5'-monophosphate dehydrogenase	EEZ41661.1	Cytoplasm
	Arginine decarboxylase catabolic	AEU10010.1	Cytoplasm
	Glutamine amidotransferases class-II (GATase)	WP_044179637.1	Cytoplasm
	Putative amidotransferase	AEU09986.1	Cytoplasm
	Diguanylate phosphodiesterase	WP_044178285.1	Membrane/ Cytoplasm
Antioxidant activity	Alkyl hydroperoxide reductase	WP_005298372.1	Cytoplasm
	Superoxide dismutase	WP_005298367.1	Periplasm
Cell envelope and wall metabolism	Lytic transglycosylase	WP_044174705.1	Outer cell membrane
	Murein transglycosylase	WP_044178572.1	Outer cell membrane
Translation, ribosomal structure and biogenesis	23S rRNA methyltransferase	WP_044175535.1	Cytoplasm
	Serine hydroxymethyltransferase	WP_005300693.1	Cytoplasm
	Alanyl-tRNA synthetase	WP_044174517.1	Cytoplasm
Cell cycle control, cell division, chromosome partitioning	Chromosome partitioning protein ParA	WP_014386679.1	Cytoplasm
Mobile and extrachromosomal element functions	Putative transposase	AEU10011.1	Cytoplasm
Other	Intramembrane serine protease GlpG	WP_044173910.1	Membrane

A total of 118 clones were selected for their reactivity with pooled adsorbed and non-adsorbed sera from convalescent *S. senegalensis* specimens after a first round of screening. In a second screening, 18 out of 118 candidate clones showed positive reaction, indicating the immunogenic character of proteins expressed during *Phdp* infection. Predicted proteins codified by inserted sequences have intracellular and membrane cell location and are involved in virulence, synthesis of intermediary products, energy metabolism and DNA replication. *In vivo* induced immunogenic proteins included inosine-5'-monophosphate dehydrogenase (Impdh) and alkyl hydroperoxide reductase (AhpC), two proteins involved in peptide synthesis: serine hydroxymethyl transferase (Shmt) and alanyl-tRNA synthetase (AlaRS) and the non-ribosomal peptide synthetase, involved in the synthesis of the siderophore piscibactin (Irp2).

Discussion

Environmental stimuli determine the gene expression of organisms. Thus, bacteria invading a host sense *in vivo* environment and adapt by inducing or repressing the expression of specific genes. In the case of pathogens, identification of genes with up-regulated expression *in vivo* compared to *in vitro* conditions might give an insight into the genes relevant to the bacterial virulence [2]. AhpC peroxidase activity has a protective role by reducing hydrogen peroxide, peroxynitrite and organic hydroperoxides. Immunization with AhpC conferred protection against *Helicobacter pylori* infection [3]. Impdh catalyzes the conversion of products essential in *de novo* synthesis of guanine nucleotides. Adequate levels of purine nucleotides are critical for cell proliferation, nucleic acid replication, cell signaling and as a biochemical energy source. This gene is an important therapeutic target against bacterial diseases [4]. On the other hand, the non-ribosomal peptide synthetase involved in the synthesis of the siderophore piscibactin is considered a virulence factor in *Phdp*. In the present work, the induction in *S. senegalensis* and its immunogenic character have been determined.

Conclusion

Different proteins expressed during *Phdp* infection in *S. senegalensis* have been identified. Among them, Impdh, AhpC, Shmt, AlaRS and Irp2 have been identified as *in vivo* induced antigens expressed during *S. senegalensis* infection with *Phdp*. They are likely to play a role in the virulence of *Phdp*. The antigenic character of these proteins makes them potential targets for the development of new vaccines.

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References

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